Name _____

Investigation 9: Biotechnology
Restriction Enzyme Analysis of DNA

Background

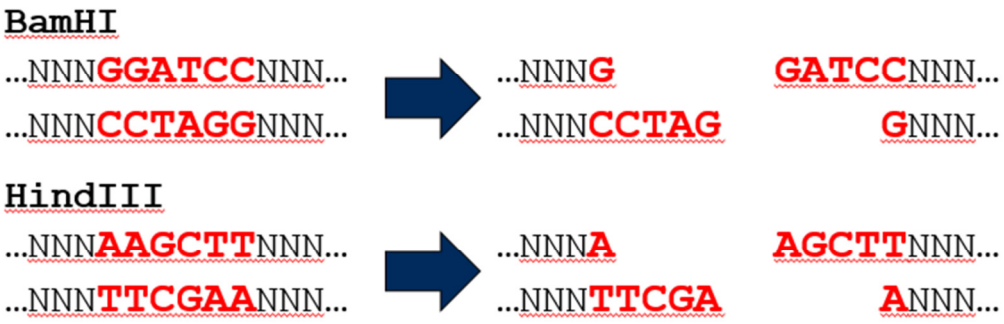
Applications of DNA profiling extend beyond what we see on television crime shows. Are you sure that the hamburger you recently ate at the local fast-food restaurant was actually made from pure beef? DNA typing has revealed that often "hamburger" meat is a mixture of pork and other nonbeef meats, and some fast-food chains admit to adding soybeans to their "meat" products as protein fillers. In addition to confirming what you ate for lunch, DNA technology can be used to determine paternity, diagnose an inherited illness, and solve historical mysteries, such as the identity of the formerly anonymous individual buried at the Tomb of the Unknown Soldier in Washington, D.C.

DNA testing also makes it possible to profile ourselves genetically-which raises questions, including *Who owns your DNA and the information it carries?* This is not just a hypothetical question. The fate of dozens of companies, hundreds of patents, and billions of dollars' worth of research and development money depend on the answer. Biotechnology makes it possible for humans to engineer heritable changes in DNA, and this investigation provides an opportunity for you to explore the ethical, social, and medical issues surrounding the manipulation of genetic information.

Restriction Enzymes

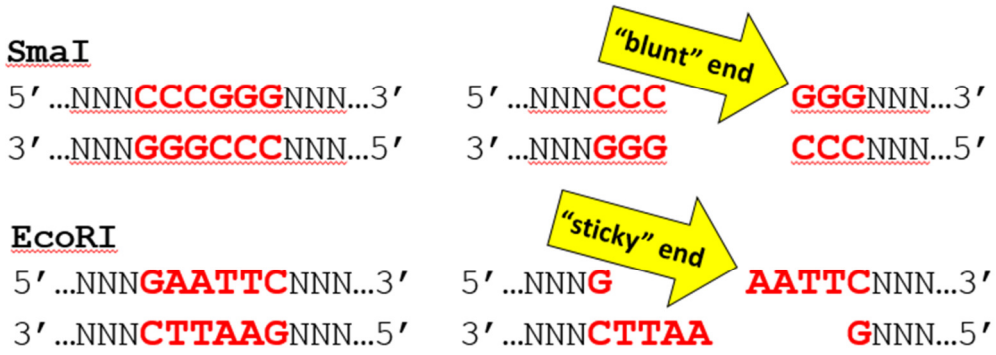
Restriction enzymes are essential tools for analyzing DNA structure, and more than 200 enzymes are now available commercially. Each restriction enzyme is named for the bacterium in which it was first identified; for example, *Eco*RI was the first enzyme purified from *Escherichia coli*, and *Hin*dIII was the third enzyme isolated from *Haemophilus influenza*. Scientists have hypothesized that bacteria use these enzymes during DNA repair and as a defense against their infection by bacteriophages. Molecular biologist use restriction enzymes to manipulate and analyze DNA sequences.

How do restriction enzymes work? These enzymes digest DNA by cutting the molecule at specific locations called restriction sites. Many restriction enzymes recognize a 4- to 10- nucleotide base pair (bp) palindrome, a sequence of DNA nucleotides that reads the same from either direction.



Name _____

Some restriction enzymes cut (or "cleave") DNA strands exactly in the center of the restriction site (or "cleavage site"), creating blunt ends, whereas others cut the backbone in two places, so that the pieces have single-stranded overhanding or "sticky" ends of unpaired nucleotides.



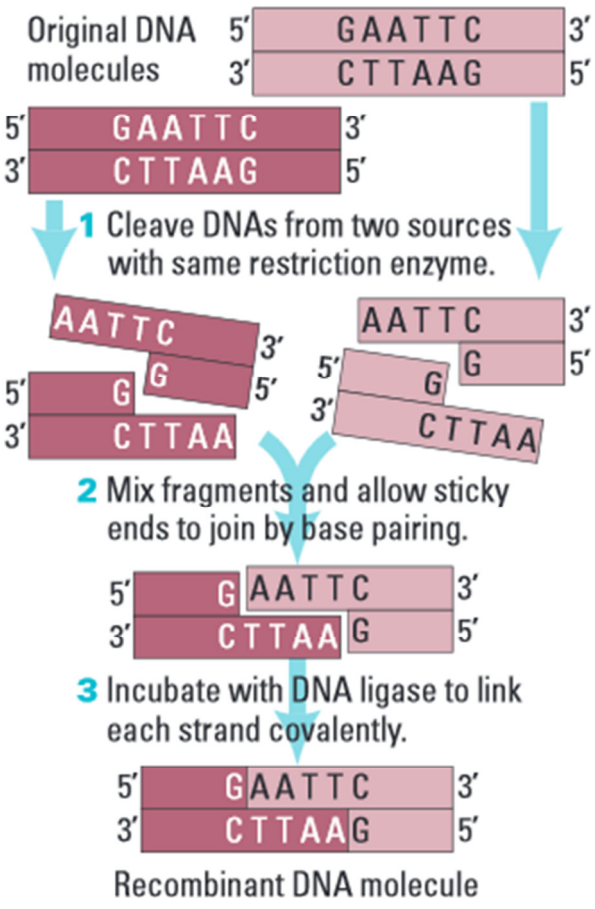


Figure 1. Recombinant DNA Using Restriction Enzymes

Two pieces of DNA that are cut with the same restriction enzyme, creating either sticky ends or blunt ends, can be "pasted" together using DNA ligase by reconnecting bonds, even if the segments originated from different organisms. An example of combining two "sticky end" sequences from different sources is shown in Figure 1. The ability of enzymes to "cut and paste" DNA fragments from different sources to make recombinant DNA molecules is the bases of biotechnology.

If bacteria produce restriction enzymes, why doesn't their own DNA get cut up? Recall that the restriction enzyme cuts DNA anywhere the recognition sequence occurs. However, it will not cut if the DNA is methylated (has $-CH_3$ groups added). So while bacteria produce restriction enzymes to cut up foreign DNA (such as the invading DNA of a virus), they also produce modification enzymes. These methylases act at the same recognition site as the restriction enzyme, protecting the bacteria's own DNA from its own restriction enzymes. This is called a "restriction-modification" system because the viral DNA is ***restricted*** in the bacterial cell by the restriction enzyme, and the bacterial DNA is ***modified*** by the methylase and thus is provided protection from its own restriction enzyme.

Name _____

DNA Mapping Using Restriction Enzymes

One application of restriction enzymes is restriction mapping. Restriction mapping is the process of cutting DNA at specific sequences with restriction enzymes, separating the fragments from each other by a process called gel electrophoresis (without pasting any fragments together), and then estimating the size of those fragments. The size and number of DNA fragments provide information about the structure of the original pieces of DNA from which they were cut.

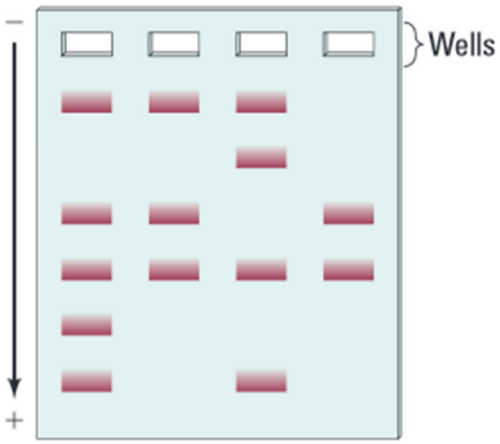
Restriction mapping enables scientists to create a genetic signature or DNA "fingerprint" that is unique to each organism. The unique fragments, called restriction fragment length polymorphisms (RFLPs), can, for instance, be used to confirm that a mutation is present in one fragment of DNA but not in another, to determine the size of an unknown DNA fragment that was inserted into a plasmid, to compare the genomes of different species and determine evolutionary relationships, and to compare samples from different individuals within a population. This latter application is widely used in crime scene investigations.

Now that you understand the basic idea of genetic mapping by using restriction enzymes, let's explore how DNA fragments can be used to make a genetic profile.

Basic Principles of Gel Electrophoresis

Creating DNA profiles depends on gel electrophoresis. Gel electrophoresis is a procedure that separates molecules on the basis of their rate of movement through a gel under the influence of an electrical field. The direction of movement is affected by the charge of the molecules, and the rate of movement is affected by their size and shape, the density of the gel, and the strength of the electrical field.

DNA is a negatively charged molecule, so it will move toward the positive pole of the gel when a current is applied. When DNA has been cut by restriction enzymes, the different-sized fragments will migrate at different rates. Because the smallest fragments move the most quickly, they will migrate the farthest during the time the current is on. Keep in mind that the length of each fragment is measured in number of DNA base pairs. Gel electrophoresis can separate DNA fragments form about 200 to 50,000 base pairs (bp).



Each fragment of DNA is a particular number of nucleotides, or base pairs, long. When researchers want to determine the size of DNA fragments produced with particular restriction enzymes, they run the unknown DNA alongside DNA with known fragment sizes. The known DNA acts as a marker.

Name _____

Pre-Lab Questions

1. You have a piece of DNA with the following template strand. What is the sequence of the complementary DNA strand? Draw it directly below the template strand.

5'-AAAGTCGCTGGAATTCACTGCATCGAATTCCCGGGGCTATATATGGAATTCGA-3'

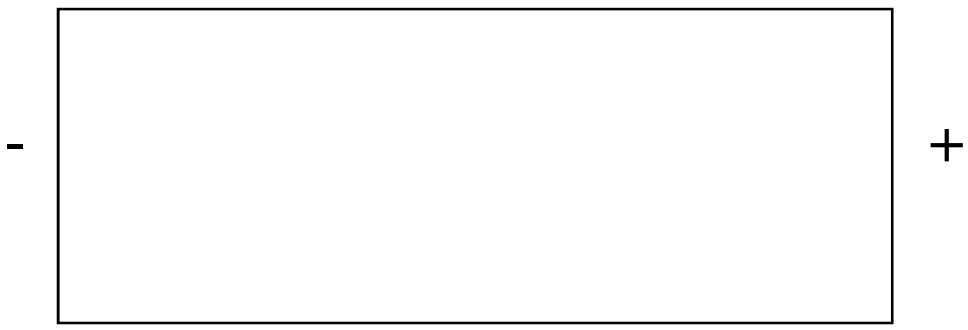
3'- -5'

2. Imagine the above segment of DNA is cut with the restriction enzyme *Eco*RI. The restriction site for *Eco*RI is 5'-GAATTC-3', and the enzyme makes a staggered ("sticky end") cut between G and A on both strands of the DNA molecule. Based on this information, draw the resulting DNA fragments.
(Hint: The restriction enzyme cuts DNA wherever its recognition site appears…and it may appear multiple times, resulting in multiple fragments)

3. The electrophoresis apparatus creates an electrical field with positive and negative poles at the ends of the gel. When you load the DNA into the wells, should the wells be oriented at the positive or negative pole and why? (Be sure to explain your answer)

4. What size fragments (large vs. small) would you expect to move the farthest through the gel? Explain.

5. A certain restriction enzyme digest results in DNA fragments of the following sizes: 4000 bp, 400 bp, 2000 bp, and 2500 bp. In the space below, sketch the resulting separation by electrophoresis. Show the location of the well where the DNA is loaded to start and the resulting bands (labeled with their respective sizes).

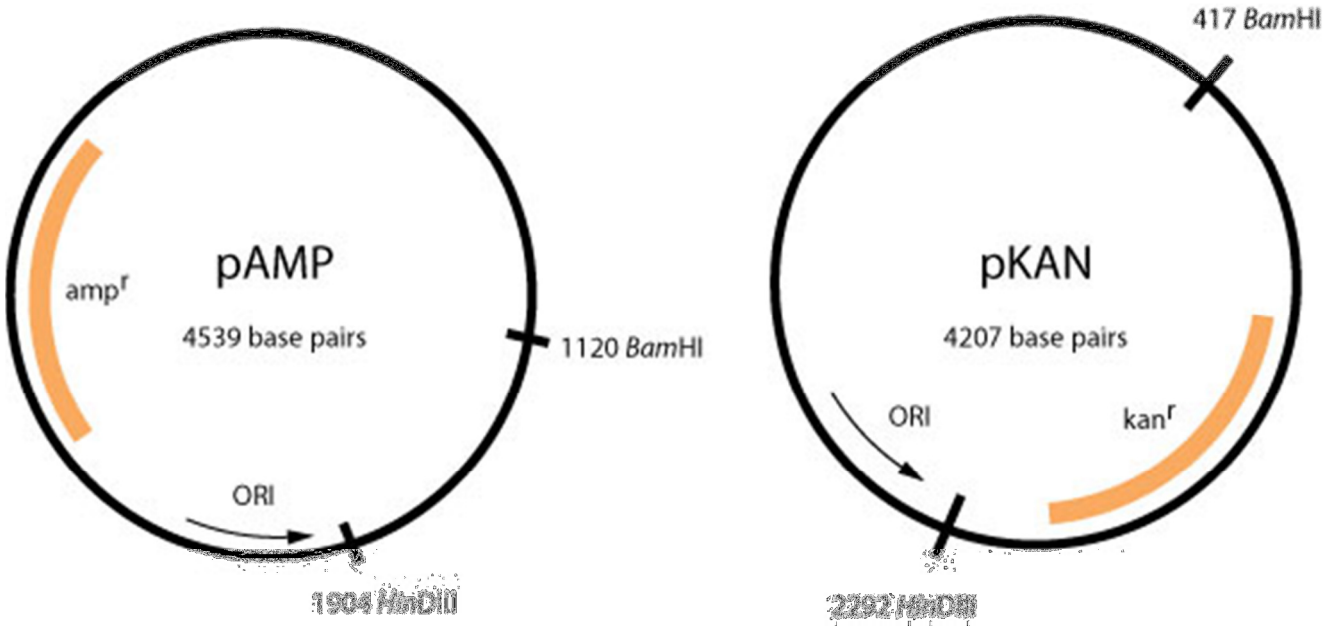


Name _____

Procedure

You will be given a sample of EITHER plasmid DNA pAMP or pKAN. Your goal is to determine which plasmid DNA you were given.

How can you tell them apart?.....Restriction mapping!



Micropipetting Review

1st Step, 1st Stop.
2nd Step, 2nd Stop.

FIRST STEP: Measuring

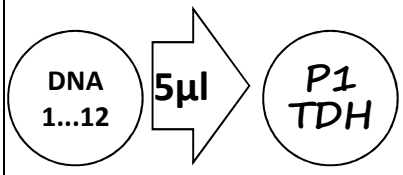
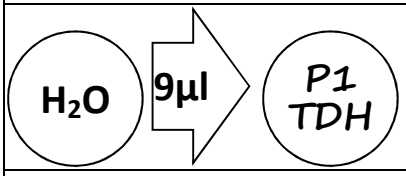
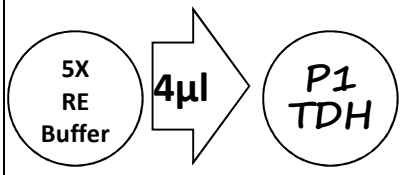
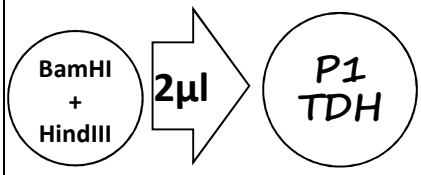
- Choose a micropipette whose range spans the desired volume.
- Set the micropipette to the desired volume by turning the plunger knob. **Don't force past the pipette's limits, as this breaks the pipette!**
- Place a tip on the micropipette, matching tip and plunger colors.
- Depress plunger to the **FIRST STOP** and HOLD.
- Place the tip into the liquid.
- Slowly release the plunger, keeping tip in liquid.

SECOND STEP: Dispensing

- Place tip against side of tube, near bottom.
- Depress plunger to the **SECOND STOP** and **HOLD**.
- Remove tip from tube **while holding down plunger**.
- Release plunger.
- Throw away used tip using the ejector button. **Use a new tip each time you pipet.**

Name _____

Session 1: Restriction Digest Reactions

	**Step 1.** Take ONE 1.5ml microcentrifuge tube from the screw-cap jar. With the black marker, label the top of the tube with: **"P" and your assigned number.** **The FIRST initials of your team members** • This tube is your **Restriction Digest** tube.
	• Your team was given a sample of *either* pAMP or pKAN plasmid DNA in a tube numbered between 1 and 12. **Step 2.** From the tube labeled with **a number between 1 and 12**, use your micropipette to measure **5µl** (microliters) of plasmid DNA and transfer it to your **Restriction Digest** tube. • At a DNA concentration of 0.1µg/µl, this 5µl will contain 0.5µg (micrograms) or 500ng (nanograms) of DNA.
	**Step 3.** From the tube labeled **H_2O**, measure **9µl** of water and transfer it to your **Restriction Digest** tube.
	• Enzymes require a chemical environment of the right pH and concentration of ions. The 5X restriction buffer is a concentrated mix that provides the environment needed for the restriction enzymes to work properly. **Step 4.** From the tube labeled **5X RE Buffer**, measure **4µl** of 5x Restriction Digest Buffer and transfer it to your **Restriction Digest** tube.
	• You will cut your plasmid DNA with two restriction enzymes: BamHI and HindIII.. **Step 5.** From the tube labeled **BamHI + HindIII** measure **2µl** of the BamHI and HindIII mix and transfer it to your **Restriction Digest** tube.
	Step 6. Close the cap on your **Restriction Digest** tube and place it in the **heating** block set at 37°C. • The restriction enzymes work best at 37°C. The reactions will incubate for one hour, then be stored in a freezer until you examine them using gel electrophoresis.

Reaction Component	Volume to Add
Your Plasmid DNA Sample (0.1µg/µl)	5µl
H_2O	9µl
5X Restriction Buffer	4µl
BamHI + HindIII Restriction Enzyme mix	2µl
Total Volume	20µl

Once your set up is incubating, determine the lengths of the fragments that will result from the restriction digest reactions and record in Table 1 of the "Analyzing your Results" section.

Name _____

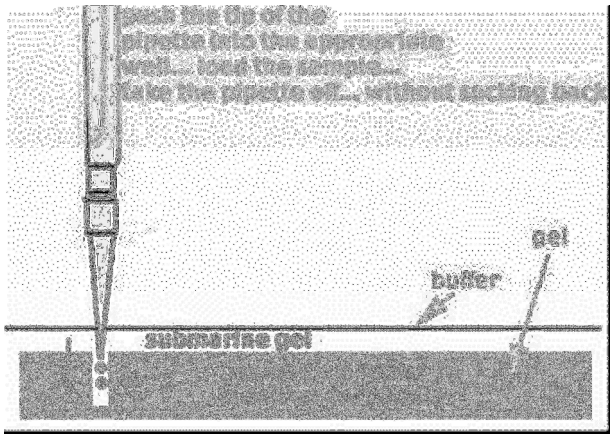
Session 2: Gel Electrophoresis

Prepare your samples for loading

1. Add 4µl of the 6X Loading Dye to your restriction digest sample.
    - If your liquids are sticking separately to the side of the tube, flick the tube with your finger and tap the bottom gently on your lab bench.

Load your sample on the FlashGel

2. When called, bring the following to the FlashGel:
    - Your DNA sample
    - Micropipette with tip
3. Slowly draw up 6 µl of your sample into the pipette (1st step, 1st stop!)
4. Using two hands, steady the pipette over the well you are going to load
5. Dip the tip of the pipette through the surface of the buffer, positioning it just inside the appropriate well (Take care not to puncture the bottom of the well with the pipette tip!)
6. Slowly depress the plunger to the 2nd stop to dispense your sample and HOLD
    - The loading dye is more dense than the buffer, causing it to sink to the bottom of the well
7. Remove the tip from the well while holding down the plunger so as not to suck the sample back into the pipette
8. Release the plunger and throw away the tip using the ejector button



Run the Gel

9. A power supply provides current to the electrodes and through the buffer and gel
10. The progress of migration through the gel is monitored with tracking dyes that are visible without the transilluminator
    - 1.2% FlashGel
    - 200 V
    - 8 minutes

Record and Analyze your Results

11. Draw the resulting bands in Figure 1, including the ladder that acts as a benchmark and the results of your classmates
12. Put a star next to the well that indicates your sample

Draw a Conclusion

13. Determine which plasmid your group had and explain how you came to this conclusion
14. Complete the extension questions to apply what you have learned from this lab

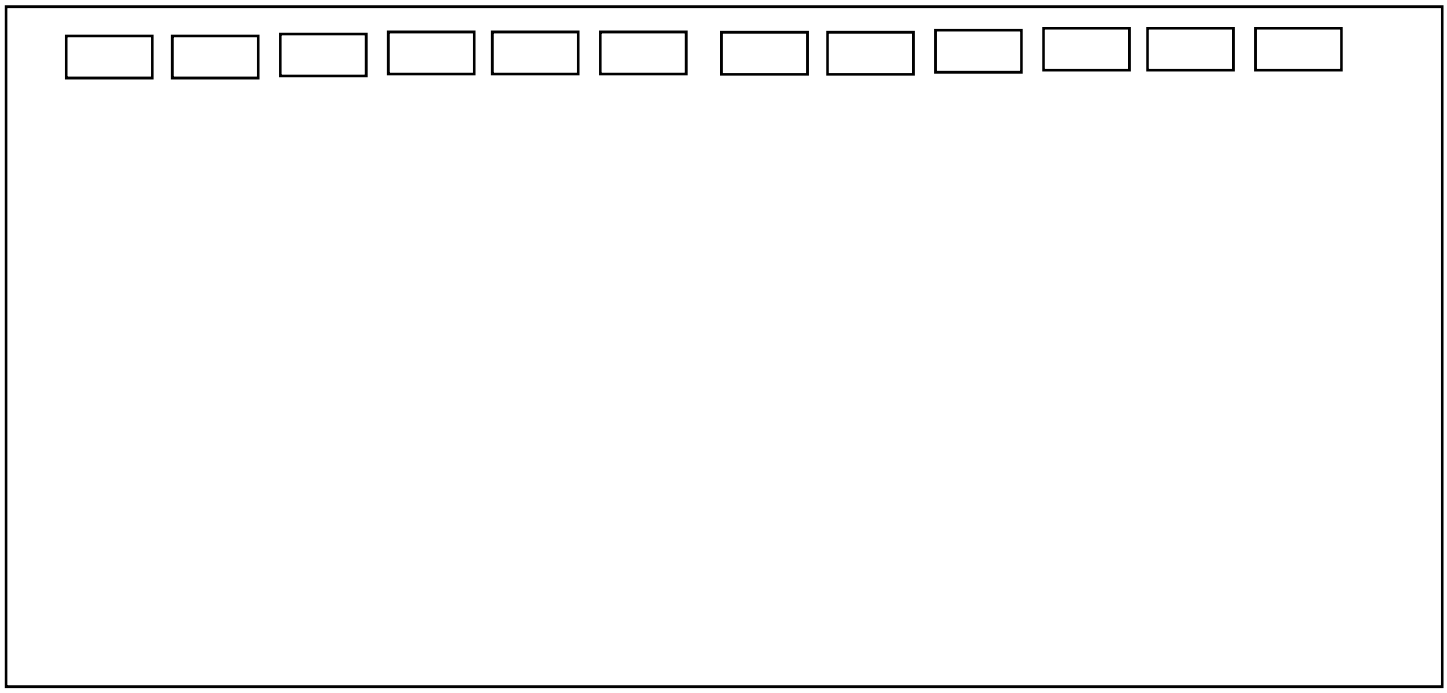
Name _____

Analyzing your Results

Table 1: Restriction Maps

Plasmid DNA	Show your Math	Size of Fragments (bp)
pAMP		
pKAN		

Figure 1: Resulting Gel



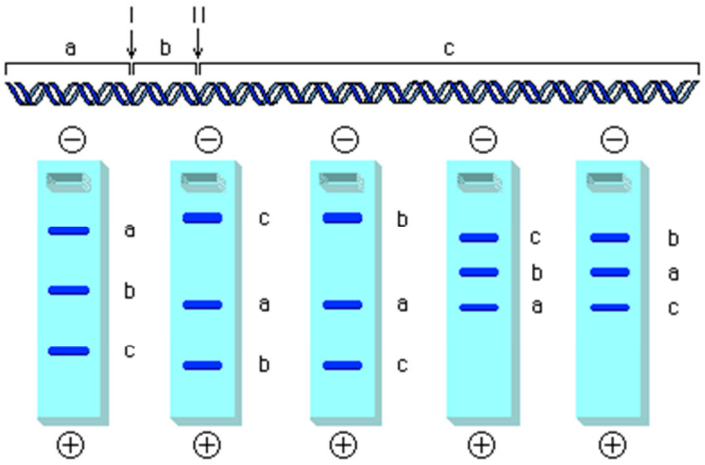
Conclusion

Which plasmid DNA was in your group's sample? Explain how you came to this conclusion.

Name _____

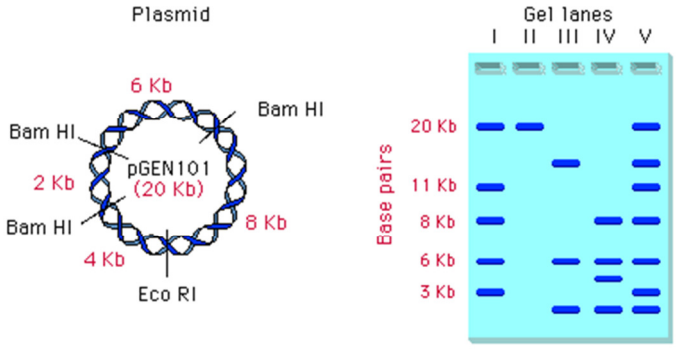
Extensions

1. A segment of DNA has two restriction sites–I and II. When incubated with restriction enzymes I and II, three fragments will be formed–a, b, and c. Indicate which of the following gels produced by electrophoresis would represent the separation and identity of these fragments by CIRCLING it.

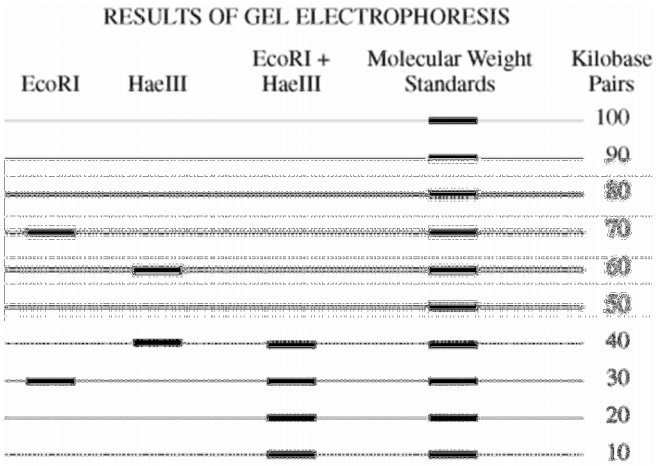


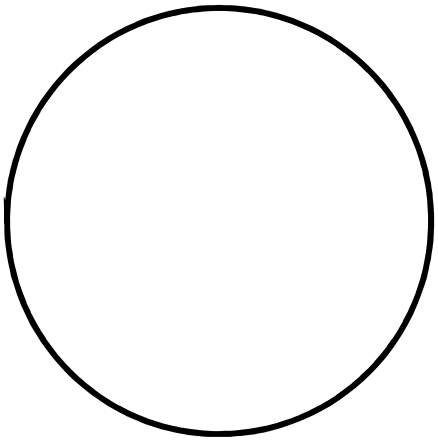
2. Below is a plasmid with restriction sites for BamHI and EcoRI. Several restriction digests were done using these two enzymes either alone or in combination.

- Which lane shows a digest with BamHI only?
- Which lane shows a digest with EcoRI only?
- Which lane shows the fragments produced when the plasmid was incubated with BOTH EcoRI AND BamH1?



3. A bacterial plasmid is 100 kb in length. The plasmid DNA was digested to completion with two restriction enzymes in three separate treatments:
EcoRI, HaeIII, and EcoRI + HaeIII (double digest).
The fragments were then separated with electrophoresis as shown. Using the circle provided, construct a labeled diagram of the restriction map of the plasmid.





Name _____

4. There are important social and ethical implications of DNA analysis. Already, DNA testing can reveal the presence of markers of certain genetic diseases, such as Huntington's.

- Explain at least 2 benefits that could result from knowing this information
- Do you feel these benefits outweigh the concern that others (including health insurance companies, college admissions offices, future employers, etc.) may somehow gain access to this information as well? Explain your stance.
- If you knew a certain genetic disease, like Huntington's, ran in your family, would you want to be tested? Why or why not?

5. With genetic engineering, biotechnicians can clip out beneficial genes from native plants in foreign countries and insert them into their crop plant relatives here in the United States.

- Explain at least 2 potential benefits that could result from such genetic engineering
- Explain at least 2 potential problems that could result
- Do you think the benefits outweigh the problems? Why or why not?